methylmercury took place either in the fish body or in the aquarium water. The difference in the percentage of inorganic Hg in surviving and non-surviving fish indicated that the transformation took place in the fish, rather than in the aquarium. The higher percentage of inorganic Hg in non-surviving fish than in surviving fish pointed to a definite correlation between biodegradation and manifestation of the toxicity of mercury, which is supported by a similar finding recently reported for methylmercury neurotoxicity in rats[9]. The increasing percentage of inorganic Hg in fish with increasing concentration of ascorbic acid in aquarium water revealed a role of vitamin C in biodegradation of methylmercury to inorganic Hg. This contention was supported by identical values for inorganic Hg in the control and ascorbic acid-treated fish in experiment III in which the lid was not placed on the aquarium and hence ascorbic acid could be oxidized by the air.

The biodegradation of methylmercury salts in rats[8] was found to be mediated by ascorbic acid, possibly by forming a free radical of ascorbate[4]. In our view the reductive cleavage of R-Hg-X compounds could occur in the presence of proton donors[10]:

$$R\text{-}Hg\text{-}X + e^- \rightarrow RHg^{\cdot} + X^-$$

$$R\text{-}Hg^{\cdot} + e^- + H^+ \rightarrow R\text{-}H + Hg$$

The table also shows that in control fish exposed to mercuric chloride, there was a surprisingly large biotransformation of $HgCl_2$ into some organic form, presumably dimethylmercury or methylmercury[11,12]. It was already known that methylation of inorganic mercury takes place in fish liver in vitro[11,12], but that it occurs in vivo and at such a high level is a new finding. The exposure of goldfish to ascorbic acid resulted in reduction of $HgCl_2$ toxicity, as evidenced by the significantly greater number of surviving fish in the majority of sub-groups ($\chi^2$ was 8.57, 7.53 and 10.0, giving $p < 0.01$). A similar effect of ascorbic acid in decreasing inorganic mercury toxicity in chicks has recently been reported[13]. In our study, the protective effect was not due to conversion of $Hg^{2+}$ into less toxic $Hg^{1+}$ or $Hg^0$ because an even greater proportion of $HgCl_2$ was found to be converted into some organic form. Therefore the suggestion that the ascorbic acid forms complexes with many biologically important elements including mercury[13], thus modifying its toxicity, is worthy of further investigation.

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## Diurnal rhythm of hemocyte population in an insect, *Schizodactylus monstrosus* Drury

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*Summary.* In *Schizodactylus monstrosus* the total hemocyte count appeared to be much lower during the day than at night. At night, the percentage of prohemocytes and spherule cells increased substantially over that of granular hemocytes, plasmatocytes and adipohemocytes. The percentage of sessile hemocytes was much higher during the day. Hemogram rhythmicity was much affected in decapitated insects; altered photoperiod had little effect on it.

Studies on insect hemocytes under various experimental conditions have received much attention due to their importance as an index of various physiological conditions[1-3]. Most animals show circadian rhythms in their behavior; circadian patterns are also reflected in the physiology of various systems in many insects[4-6]. Biochemical changes of the hemolymph are reported to occur following a definite diurnal periodicity[7,8]; similar rhythms in the mitotic activities of hemocytes have also been reported in some insects[9-11]. From various studies it is evident that all hemocytes do not circulate at the same time, some remain adhering to tissue surfaces; their appearance in and disappearance from the circulation also seems to follow a definite rhythm[12,13]. In view of these facts, the present investigation attempts to report the changes in the hemogram of a nocturnal, sand burrowing insect, *Schizodactylus monstrosus* (Orthoptera, Schizodactylidae) during different hours of day and night under normal and experimental conditions.

To avoid the effects of varying reproductive cycles on the hemocyte population in females, only adult males were used in this investigation. After collection, insects were kept separately in moist sand jars under a) complete darkness (maintaining natural conditions) and b) altered photoperiodic conditions subjecting them to 12 h of light and 12 h of dark conditions. Observations on the total hemocyte count (THC), differential hemocyte count (DHC) and hemolymph volume were made every 4 h during the day and every 2 h during the night (because of their nocturnal behavior). Hemolymph samples were collected by amputating one of the 3rd legs. For THC, hemolymph was allowed to fill the Neubauer hemocytometer chambers by capillary action about 5 sec after leg amputation. No anticoagulant was used, to avoid errors in counting; gelification of plasma in *Schizodactylus monstrosus* occurs at room temperature at about 200–210 sec after blood is shed. DHC was made by staining air-dried blood films with Leishman's stain. Cell size, position of the nucleus, nature of cytoplasmic inclusions and staining reactions were used as the main criteria for identification of hemocyte types.

To evaluate the proportions of free and sessile hemocytes

during different hours of the day and night, THC and DHC were made from heat fixed insects by subjecting them to 50 °C for 5 min. Temperature stress drives the hemocytes from their temporary reservoirs into the circulation. To assess the role of hormones, insects were decapitated following the technique of Wigglesworth[14]. Observations under altered photoperiodic conditions were made by subjecting the insects to L:D = 12:12 condition. THC, DHC and Blood volume changes were recorded under all these experimental conditions at the same intervals as in the control.

For the estimation of hemolymph volume, insects were weighed and injected with 1 µl of 2% Congo red dye, which was allowed to circulate for 5 min after which 1 µl of the dyed blood was drawn out and diluted to 1 ml with insect saline. Transmission readings of this dyed blood were compared with that of the undyed blood serving as control. Blood volume was estimated by plotting the data using Lee's equation[15]. For each experiment single specimes were used.

THC remained relatively uniform ($16\text{-}18 \times 10^3$ cells/mm$^3$) from 06.00 h. to 18.00 h. Subsequently the count showed a peak of $28.7 \times 10^3$ cells/mm$^3$ at 22.00 h followed by a steady decline (table 1). Hemocyte categories comprised prohemocytes, plasmatocytes, granular hemocytes, adipohemocytes and spherule cells[3]. During the day these comprised 18-21%, 12-15%, 43-49%, 15-22% and 2-3% of the hemocyte population respectively (table 2). Prohemocytes and granular hemocytes showed a 28-35% and 6-9% increase respectively between 18.00 h and midnight compared to a 17-31% decrease of granular hemocytes during the same period; percentages of plasmatocytes and adipohemocyte did not alter significantly.

Temperature stress resulted in a 29-31% increase in THC during the day against only 7-11% during the night (table 1). Prohemocytes, plasmatocytes, granular hemocytes and adipohemocytes exhibited 55-58%, 23-29%, 10-12% and 1-11% increase respectively during the night (table 2). Heat shock had an insignificant effect on the numerical variations of spherule cells.

Decapitation markedly increased THC and the percentage of prohemocytes and plasmatocytes with simultaneous variations in the periodicity of the hemogram (tables 1 and 2). Under altered photoperiodic conditions, 12 h sustained light during the day resulted in an insignificant decrease in THC and the percentage of prohemocytes and plasmatocytes; also alterations in other cell counts appeared to be insignificant (tables 1 and 2).

In all the experimental conditions THC and DHC appeared not to be related to the changes in hemolymph volume, since the latter varied from 278 to 296 µl/insect.

The total number of hemocytes and the appearance of specific cell types in the circulation following a definite periodicity demonstrates a rhythm in the hemogram. Similar diurnal rhythms have been reported in a cockroach, *Blaberus giganteus*[12] and in a crab, *Emerita asiatica*[13]. In *Schizodactylus monstrosus*, periodicity of the apperance and disappearance of hemocytes in and from the circulation is seen to coincide closely with the normal nocturnal behavior

Table 1. Total hemocyte count (THC; data $\times 10^3$ cells/mm$^3$) and hemolymph volume (Hm.V.; µl/insect) in *S. monstrosus* in control, heat-treated and decapitated insects and under altered photoperiod conditions during different hours of the day and night

| | | 06.00 h | 10.00 h | 15.00 h | 18.00 h | 20.00 h | 22.00 h | 24.00 h | 02.00 h | 04.00 h |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | THC | 16.2 ±0.12 | 16.9 ±0.15 | 17.5 ±0.13 | 18.0 ±0.12 | 26.7 ±0.18 | 28.7 ±0.17 | 25.5 ±0.15 | 21.1 ±0.18 | 18.2 ±0.12 |
| | Hm.V. | 280 ±6 | 283 ±4 | 281 ±8 | 279 ±5 | 285 ±3 | 280 ±8 | 284 ±9 | 278 ±5 | 281 ±4 |
| Heat-treated | THC | 20.9* ±0.20 | 21.9 ±0.15 | 22.7* ±0.21 | 23.3* ±0.17 | 28.6** ±0.12 | 31.0** ±0.16 | 27.5** ±0.13 | 23.2 ±0.13 | 20.2* ±0.19 |
| | Hm.V. | 289 ±7 | 288 ±3 | 294 ±7 | 285 ±8 | 296 ±9 | 291 ±6 | 290 ±9 | 289 ±8 | 288 ±5 |
| Decapitated | THC | 22.6 ±0.17 | 24.5* ±0.13 | 21.2 ±0.15 | 23.7* ±0.21 | 28.5** ±0.19 | 29.9 ±0.11 | 28.7** ±0.13 | 27.2 ±0.14 | 29.7** ±0.20 |
| | Hm.V. | 283 ±8 | 289 ±5 | 278 ±7 | 289 ±9 | 284 ±8 | 281 ±6 | 283 ±7 | 280 ±6 | 279 ±9 |
| Altered photoperiod (L:D = 12:12) | THC | 14.8 ±0.1 | 15.0 ±0.11 | 15.9 ±0.1 | 18.1 ±0.11 | 25.9 ±0.1 | 28.8 ±0.12 | 25.7 ±0.1 | 21.2 ±0.12 | 19.5 ±0.11 |
| | Hm.V. | 281 ±7 | 287 ±5 | 286 ±3 | 282 ±9 | 281 ±6 | 280 ±5 | 284 ±8 | 287 ±9 | 289 ±6 |

Values represent mean ± SE (replication No. 11). Significant at *p = 0.01 level; **p = 0.05 level.

Table 2. Differential hemocyte count (expressed as %) in *S. monstrosus* under control (C), temperature treated (T), decapitated (D) and altered photoperiod (P) conditions during different times of day and night

| | Prohemocytes | | | | Plasmatocytes | | | | Granular hemocytes | | | | Adipohemocytes | | | | Spherule cells | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | C | T | D | P | C | T | D | P | C | T | D | P | C | T | D | P | C | T | D | P |
| 06.00 h | 18 | 27 | 25 | 16 | 14 | 16 | 18 | 13 | 46 | 39 | 32 | 48 | 20 | 16 | 23 | 21 | 2 | 2 | 2 | 2 |
| 10.00 h | 20 | 31 | 23 | 17 | 15 | 19 | 19 | 14 | 46 | 32 | 37 | 48 | 18 | 17 | 19 | 20 | 1 | 1 | 2 | 2 |
| 14.00 h | 19 | 30 | 24 | 18 | 14 | 18 | 16 | 13 | 43 | 35 | 30 | 44 | 22 | 16 | 29 | 23 | 2 | 1 | 1 | 1 |
| 18.00 h | 21 | 33 | 28 | 20 | 13 | 17 | 15 | 13 | 45 | 30 | 31 | 46 | 18 | 18 | 24 | 18 | 3 | 2 | 2 | 2 |
| 20.00 h | 35 | 40 | 26 | 34 | 21 | 22 | 17 | 20 | 30 | 33 | 27 | 28 | 10 | 23 | 28 | 10 | 4 | 2 | 2 | 4 |
| 22.00 h | 38 | 44 | 25 | 37 | 21 | 24 | 18 | 20 | 16 | 20 | 39 | 15 | 12 | 10 | 17 | 11 | 3 | 2 | 1 | 3 |
| 24.00 h | 39 | 36 | 29 | 38 | 24 | 25 | 16 | 24 | 20 | 20 | 33 | 21 | 14 | 17 | 20 | 14 | 3 | 2 | 2 | 3 |
| 02.00 h | 29 | 32 | 28 | 28 | 18 | 21 | 19 | 17 | 34 | 33 | 30 | 36 | 17 | 22 | 22 | 17 | 2 | 2 | 1 | 2 |
| 04.00 h | 22 | 25 | 30 | 21 | 16 | 20 | 18 | 15 | 39 | 40 | 30 | 41 | 22 | 24 | 20 | 22 | 1 | 1 | 2 | 1 |

Values are means of 500 cells.

of the insect, since they spend the whole day in their burrows (90–120 cm below the sand) and come out at 2 h after sunset (personal observation). Hemocytological features seem to indicate that this strictly nocturnal activity brings about important physiological changes which induce these hematological alterations.

Increased THC from 18.00 h to 22.00 h may be due to synchronized mitosis of the undifferentiated hemocytes[16] or release of sessile hemocytes from the temporary hemocyte reservoirs (indicated by the differences in THC in normal and heat fixed specimens during the period). Effects of heat shock on THC and DHC seem to indicate that during the day a majority of the hemocytes remain out of circulation, prohemocytes and granular hemocytes comprising the bulk of these cells[17].

Hemocyte periodicity was greatly altered in decapitated insects, which is indicative of the fact that neurohormones play a key role in maintaining the rhythmicity, since with the removal of the brain neurosecretory cells are eliminated. It seems also plausible that light may be the main signal to trigger the neurosecretory cells, releasing specific hormones that bring about chemical changes in the hemolymph which in turn determine the appearance and disappearance of hemocytes in and from the circulation.


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## Different response of alfalfa plants to artificial defoliation and to feeding by pea leaf weevil (*Sitona lineatus* L.)


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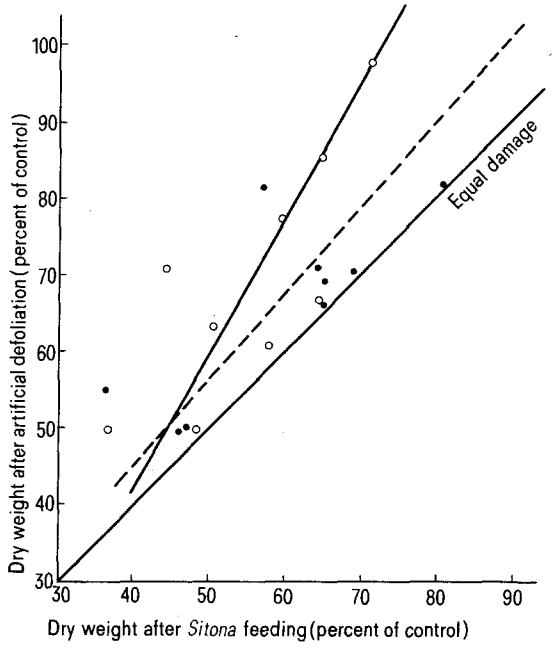
*Summary.* The consequences of defoliation of alfalfa seedlings by *Sitona lineatus* L. and by hand cutting of the same shape and extent were compared. Insect feeding caused (after 40 days) significantly greater decrease of plant weight (especially in roots) than artificial defoliation.


Some information concerning the toxic influence of sucking insects on plants is available in the literature[2,3]. Little is known about similar effects of chewing insects. The negative effects of these insects on plants is mostly attributed to the loss of plant tissue only. However, some recent works demonstrated that mechanical defoliation and defoliation by chewing insects evoked different responses in plants[4,5]. We compared the effect of hand defoliation with that caused by the feeding of pea leaf weevil on alfalfa plants.

In our experiments 9 strains derived from 5 cultivars of alfalfa were used. The seeds were sown into 3 pots (A–C) filled with a mixture of sand and peat. In each pot the plants were arranged in 9 parallel rows, every row containing 20 seeds of one strain. The plants were cultivated in greenhouse conditions. At the cotyledon stage, 90 starved females of pea leaf weevil (*Sitona lineatus* L.) were placed in the pot A, and allowed to feed for 3 h. Immediatelly after feeding, the plants in the pot B were mechanically defoliated by scissors so that in every individual plant the damage caused by the scissors closely resembled in position, shape and extent the damage caused by the beetles to the corresponding plant in the pot A. The plants in the pot C served as a control. All plants were harvested 40 days after sowing (at this time the control plants had reached the stage of the 6th true leaf), and the dry matter of the parts above ground and roots was weighted. The dry weight of damaged plants was expressed as a percentage of the dry weight of control plants.

In all strains the dry weight of artificially defoliated plants was higher than that of plants damaged by beetles (fig.).




The comparison of alfalfa response to artificial and *Sitona* beetle defoliation. The dry weight (expressed as percent of the dry weight of control) of plants after *Sitona* feeding (abscissa) plotted against dry wt of plants after artificial defoliation (ordinate). Mean values (of 20 plants) for above-ground parts (●, – – –) and roots (○, ——) of 9 alfalfa strains.